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Actinomycete communities in earthworm guts and surrounding soil

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Summary. The gut actinomycete flora of the earthworms *Lumbricus rubellus* and *Octolasion montanum* and soil, examined in the autumn, was mostly composed of *Streptomyces* spp., among which *S. diastatochromogenes* and *S. nogalater* predominated. A relatively high density of *Micromonospora* was found in the gut of *L. rubellus* but not in *O. montanum* and surrounding soil. A high proportion of streptomycete strains isolated from earthworm guts produced antibiotics active against *Bacillus subtilis* and/or *Saccharomyces cerevisiae*. No gut-strains were, however, active against *Escherichia coli*.

Key words: Earthworms, *Lumbricus rubellus*, *Octolasion montanum*, *Streptomyces*, *Micromonospora*, gut-microflora

Introduction

The composition of microbial communities in the gut contents and casts of earthworms, as well as the processes of microbial selection occuring in the worm gut have attracted the attention of both microbiologists and soil zoologists for a long time. Many papers dealing with this topic have been written recently and reviewed by Satchell (1983) and Lee (1985). Several authors have shown that actinomycetes are able to grow quickly in the worm gut and to form one of the most dominant components of the intestinal microflora of earthworms (Stöckli, 1928, Ruschmann, 1953, Schütz & Felber, 1956, Parle, 1963, Ravasz et al., 1986). Nevertheless, little is known about the ecology, biochemical characteristics and species composition of earthworm gut actinomycete communities, though this information is necessary for a better understanding of the processes which occur in the earthworm gut and for the detailed explanation of the earthworm's role in the soil. Streptomycetes are well-known as a rich source of antibiotics. The failures to detect antibiotics in soil are the reason why exist still many gaps in our knowledge of the roles played by actinomycetes in soil processes (Goodfellow & Williams 1983). If antibiotics are produced by streptomycetes (or by other microbes) in soil, they could still be of biological significance to the producers at critical stages of competition in their temporally and spatially restricted growth periods (Williams 1982). Possible antibiotic activity of streptomycetes in the soil and guts of earthworms raises the question to what extent, if any, it might be significant in determining the composition of the microbial community in those sites. Contreras (1980) and Ravasz et al. (1986) showed that in the intestinal milieu usually only few actinomycete species form large populations, while many others occur sporadically. In a previous study (Krištůfek et al. 1990) we studied soil streptomycete communities and those in the guts of two

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earthworm species belonging to different ecological groups (*Lumbricus rubellus*, *Octolasion montanum*) during spring and summer. Relatively similar streptomycete communities were found in soil and earthworm guts in spring; however, marked differences were recorded in summer. The aim of the present study was to enlarge our knowledge of the composition of actinomycete communities in the guts of those earthworms and in surrounding soil during autumn.

Materials and Methods

The sampling site was a meadow-like fallow area situated near Chelčice village, South Bohemia, Czech Republic. The soil was an entric cambisol (humus form mull, pH 5.53, C 2.74%). Two earthworm species, *Lumbricus rubellus* Hoffmeister, 1843 (epigeic) and *Octolasion montanum* (Wessely, 1905) (endogeic) were chosen for the study of gut actinomycete microflora.

Both soil samples and earthworms were collected on 11th October 1989. The soil was taken from the 0 – 10 cm layer. For sampling gut contents, 5 individuals of both earthworm species were used, obtained by hand sorting of soil samples. Each individual was tied with thread on both sides of the body, surface disinfected and washed in sterile water. After cutting off the body tail, worms were gently massaged by sterile forceps to obtain fresh gut content material.

Samples of gut content material and soil were homogenized separately. Each homogenate was suspended in sterile water, serially diluted to 10^{-4} and 0.1 ml of chosen dilution was placed on two different agar media (glycerol-casein and inorganic salts-starch agar) using spread plate method. Actinomycete colonies were randomly isolated after 14 d incubation at 28 °C. In total 446 isolates were obtained and grouped on the basis of their morphological properties. From each group, representative actinomycete strains (78) were selected for subsequent taxonomic investigations. Cell-wall analyses were made using extracts of mycelium hydrolyzates (Hasegawa et al. 1983). Standard solutions of amino-acids and sugars were chromatographed on Whatman paper No. 1 with the following solvent systems: methanol-water-hydrochloric acid-pyridine, 32 : 7 : 1 : 4 (Becker et al. 1964) for amino-acids, and butanol-pyridine-water-acetic acid, 60 : 40 : 30 : 3 (Becker et al. 1965) for whole-cell sugars. Strains of *Streptomyces* were assessed using the methods and criteria of ISP (Shirling & Gottlieb 1966). The key published by Szabó et al. (1976) was used for species determination.

The numbers of bacteria and actinomycetes on glycerol-casein agar and inorganic salts – starch agar were counted after 9 d incubation (28 °C) and expressed per 1 g dry matter. The fresh weight of gut content samples used for dilution (10^4) was: *L. rubellus* – 0.111 g, *O. montanum* – 0.268 g. Antibiotic activity of isolated strains (solid cultures) was assayed by the agar plug method using *Bacillus subtilis* CCM 1718, *Escherichia coli* CCM 2024 and *Saccharomyces cerevisiae* VŠCHT 312/1 as test organisms. The size of the inhibition zone was measured after 18 h incubation at 28 °C.

Results

The total number of microbes was found to be higher in the gut contents of *Lumbricus rubellus* than in those of *Octolasion montanum* and in the soil, for both media. A higher

Table 1. Numbers [mean (SE) of colony forming units $\times 10^6$ g^{-1} dry matter] of bacteria (*B*) and actinomycetes (*A*) in samples of gut contents of *L. rubellus* and *O. montanum* and surrounding soil

	Plating medium					
	Inorganic salts-starch agar			Glycerol-casein agar		
	B	*A*	*A* (%)	*B*	*A*	*A* (%)
Soil (0 – 10 cm)	3.78 (0.64)	2.16 (0.47)	36.4	9.53 (0.98)	1.60 (0.42)	14.4
L. rubellus	49.13 (3.44)	1.91 (0.10)	3.7	32.50 (1.11)	1.88 (0.13)	5.5
O. montanum	4.35 (0.44)	1.56 (0.10)	26.4	5.29 (0.95)	1.63 (0.12)	23.6

Table 2. Percentage frequency of *Streptomyces* species and *Micromonospora* strains isolated from the soil and gut content of earthworms

Organism	Soil	Earthworms	
	(0 – 10 cm)	*L. rubellus*	*O. montanum*
S. albofaciens	3	–	–
S. antibioticus	–	5	–
S. antimycoticus	6	–	–
S. atratus	–	–	1
S. atrofaciens	1	–	2
S. badius	–	5	–
S. chromofuscus	3	–	–
S. diastatochromogenes	21	9	30
S. endus	3	–	2
S. flavovirens	1	–	3
S. gannmycicus	1	–	–
S. griseoincarnatus	–	–	2
S. griseus	–	4	–
S. halstedii	–	3	–
S. lucensis	–	–	2
S. melanosporofaciens	3	–	6
S. nigrescens	–	8	2
S. nogalater	15	15	15
S. pilosus	1	–	–
S. phaeochromogenes	2	–	–
S. rochei	–	–	3
S. spheroides	–	3	–
S. tanashiensis	–	–	2
S. umbrosus	2	–	–
S. violascens	8	–	2
Micromonospora strains	1	13	–
Heterogenous group	29	35	28
Total number of isolates obtained	195	76	175
Total number of selected representative strains	34	16	28

proportion of actinomycetes was recovered from the gut of *O. montanum* and from the soil than from *L. rubellus* (Table 1).

The species composition of the soil and gut actinomycete floras is shown in Table 2. *Streptomyces* species seem to be the most important colonizers of both the soil and earthworm guts. A higher density of *Micromonospora* was found in gut contents of *L. rubellus* (13%) than in the soil (1%). No *Micromonospora* was isolated from the gut of *O. montanum*. *Streptomyces diastatochromogenes* and *S. nogalater*, frequently isolated from the soil, were also dominant in the gut of earthworms. All other species were found in much smaller numbers. Seven species of actinomycetes were found both in the soil and in the gut of *O. montanum*, only three of them, however, were also isolated from the gut of *L. rubellus*. Five species were isolated exclusively from the gut of *L. rubellus* and the same number of species specific to *O. montanum* was found.

Table 3 shows a comparison between antibiotic activities of streptomycete isolates obtained from the soil and those from earthworm guts. Relatively high proportions of isolates from both soil and earthworm guts were active against *Bacillus subtilis* and *Saccharomyces cerevisiae*, i.e. representatives of Gram-positive bacteria and microfungi respectively. No isolates from the earthworm guts showed any activity against *Escherichia coli* but 19% of those from the soil were active.

Table 3. Antibiotic activity of isolates of streptomycetes inhabiting earthworm guts and surrounding soil

	Number of isolates tested (100%)	Activity against		
		B. subtilis (%)	*E. coli* (%)	*S. cerevisiae* (%)
Soil (0 – 10 cm)	138	65	19	36
L. rubellus	39	61	0	28
O. montanum	126	70	0	41

Discussion

Lumbricus rubellus is known to be a consumer of materials rich in relatively undecomposed plant remains (Piearce 1978). These remains support generally higher numbers of microorganisms than mineral soil, which is the main source of food for *Octolasion montanum*. Hence it is not surprising that much higher numbers of bacteria were found in the gut of *L. rubellus* than in *O. montanum* and or in surrounding soil. Proportions of actinomycetes in the gut of *O. montanum* were similar to those in the soil. Markedly lower proportions of actinomycetes in the gut of *L. rubellus* can probably be explained by the usually low occurence of actinomycetes in undecomposed plant debris. This corresponds with the findings of Ravasz et al. (1986) who found twice as many bacteria but fewer actinomycetes in *Lumbricus polyphemus* (Fitzinger, 1833) than in *O. montanum*. Though *L. polyphemus* is an anecic earthworm, the food sources of this species and *L. rubellus* are rather similar. Presumably, the differences in feeding biology of the two earthworm species studied (Lee 1985) are the main reason for the contrast in actinomycete communities found in their guts (Table 2). The relatively high proportion of *Micromonospora* strains found in the gut flora of *L. rubellus* in contrast to their absence in the gut of *O. montanum* appears to be the most obvious difference. Among streptomycetes, two species were found to be dominant in earthworm guts, of which *Streptomyces diastatochromogenes*, an extraordinarily common widespread species (Elesawy & Szabó 1981), was more frequent in *O. montanum* than in *L. rubellus*, while *S. nogalater* was equally common in the guts of both earthworm species. This corresponds well with the results of Contreras (1980), Ravasz et al. (1986) and Krištůfek et al. (1990), which showed that only a few gut actinomycete populations occupy a prominent position in the intestinal milieu of these soil animals.

Krištůfek et al. (1990) described streptomycetes occuring in the gut contents of earthworms and in surrounding soil in spring and summer. They found that *S. diastatochromogenes*, *S. umbrosus* and *S. pilosus* were the most common species in the soil (as in this study), whereas the intestinal community of *L. rubellus* was mainly composed of *S. misakiensis*, *S. flavovirens* and *S. nigrifaciens*, and *S. nogalater* were clearly predominant in the gut of *O. montanum*. It seems that during the autumn, when earthworms are active, the differences between the actinomycete community in the earthworm gut and in surrounding soil are not so contrast as in summer, but they do exist, especially in the case of *L. rubellus*.

Antibiotic activity of actinomycetes inhabiting earthworm guts has previously been reported only by Ravasz et al. (1986), who used tests against *B. subtilis* and *E. coli*. They found that 44 of the 260 isolates tested were active against Gram-positive bacteria but the inhibition of Gram-negative bacteria was minimal (2 active strains). Szabó (1974) studied antagonism between actinomycete and bacterial strains freshly isolated from the gut content and feaces of *Bibio marci* (Linnaeus, 1758) larvae. Many strains of *Streptomyces finlayi* (Szabó et al. 1963) Pridham 1970 (highly predominant species) inhibited the growth of *B. subtilis*. Merely one culture was found to be active against Gram-negative bacteria. Similarly, Pisano et al. (1986) demonstrated that actinomycetes isolated from marine sediments were primarily active against Gram-positive bacteria, than against Gram-negative

ones or against fungi. In our work we compared the bioactivity of streptomycete isolates from earthworm guts and those from the soil. More streptomycete isolates were active against Gram-positive bacteria than against fungi in both habitats. In accordance with the observations of Ravasz et al. (1986) no activity of gut isolates was detected against Gram-negative bacteria. However, there were streptomycetes active against *E. coli* in the surrounding soil. *Streptomyces diastatochromogenes* was active against Gram-positive bacteria but not against Gram-negative bacteria or fungi, while *S. nogalater* was active against fungi but not against bacteria. Thus, it is unlikely that the streptomycete population would be able to cause any depressive antibiotical effect on the growth of the bacterial flora in earthworm guts composed mainly of Gram-negative organisms. Further studies are needed to clarify the reasons for this phenomenon.

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Appendix. List of *Streptomyces* species found during this study
S. albofaciens Thirumalachar et Bhatt 1960
S. antibioticus (Waksman et Woodruff 1941) Waksman et Henrici 1948
S. antimycoticus Waksman 1957
S. atratus Shibata et al. 1962
S. atrofaciens Ehrlich et al. 1963
S. badius (Kudrina 1957) Pridham et al. 1958
S. chromofuscus (Preobrazhenskaya et al. 1957) Pridham et al. 1958
S. diastatochromogenes (Krainsky 1914) Waksman et Henrici 1948
S. endus Anderson et Gottlieb 1952
S. flavovirens (Waksman 1923) Waksman et Henrici 1948
S. ganmycicus Hosoya et Soeda 1955
S. griseoincarnatus (Preobrazhenskaya et al. 1957) Pridham et al. 1958
S. griseus (Krainsky 1914) Waksman et Henrici 1948
S. halstedii (Waksman et Curtis 1916) Waksman et Henrici 1948
S. lucensis Arcamone et al. 1959
S. melanosporofaciens Arcamone et al. 1959
S. nigrescens (Sveshnikova 1957) Pridham et al. 1958
S. nogalater Bhuyan et Dietz 1966
S. pilosus Ettlinger et al. 1958
S. phaeochromogenes (Conn 1917) Waksman 1957
S. rochei Berger et al. 1949
S. spheroides Wallick et al. 1956
S. tanashiensis Hata et al. 1952
S. umbrosus Schmidt-Kastner et al. 1957
S. violascens (Preobrazhenskaya et Svenshnikova 1957) Pridham et al. 1958